



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF BREAST CYST FLUID ON OESTRONE SULPHATASE
ACTIVITY IN BREAST CANCER CELLINES**

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**EFFECT OF BREAST CYST FLUID ON OESTRONE SULPHATASE
ACTIVITY IN BREAST CANCER CELL LINES**

By

RASHA EL-HAG EL-SADIG

**Thesis Submitted in Fulfilment of the Requirements for the Degree of Master of
Science in the Faculty of Medicine and Health Sciences
Universiti Putra Malaysia**

September 2000



Especially to

My mother, brother, sister, and Memories of the greatest Dad and loving brother



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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Chairman: Professor Dr. Leslie C Lai

Faculty: Medicine and Health Sciences

Breast cancer is the most common cancer in women worldwide. Although breast cancers are generally oestrogen receptor positive initially, a substantial proportion become oestrogen receptor negative. Oestrogen receptor positive breast cancers are associated with a better prognosis than oestrogen receptor negative breast cancers as they are more responsive to hormonal therapy.

Breast cyst fluid (BCF) is known to be a rich source of steroid hormones and growth factors. These substances may have significant effects on mammary epithelial cell growth and oestrogen metabolism in the breast and may, thus, play important roles in the pathophysiology and development of breast cancer. Oestrone sulphate acts as a large reservoir of active oestrogens in the breast and is converted to oestrone by the enzyme oestrone sulphatase. The aims of the present study were to assess the effects of BCF on cell growth of, and oestrone sulphatase activity in the MCF-7 oestrogen

receptor positive and MDA-MB-231 oestrogen receptor negative breast cancer cell lines and to determine the molecular weights of the proteins/peptides in BCF responsible for the inhibition of oestrone sulphatase activity.

MCF-7 and MDA-MB-231 cell growth were significantly inhibited by BCF. Oestrone sulphatase activity was significantly inhibited in the MDA-MB-231 cells while oestrone sulphatase activity was significantly stimulated in MCF-7 cell by BCF. Oestrone sulphatase activity was significantly inhibited by both dialysed BCF and dialysate.

The presence of endogenous inhibitors of oestrone sulphatase in BCF is important because inhibitors of oestrone sulphatase are potential agents for the treatment of hormone-dependent breast cancers. The substance present in BCF, which corresponds to a molecular weight of around 28 kDa, may possibly have a role to play in the prevention of breast cancer development and progression.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains.

**KESAN BENDALIR SISTA PAYUDARA KEATAS AKTIVITI ESTRON
SULFATASE PADA TITISAN SEL BARAH PAYUDARA**

Oleh

RASHA EL-HAG EL-SADIG

September 2000

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Barah payudara adalah barah yang paling kerap terjadi dikalangan wanita di seluruh dunia. Walaupun pada peringkat awalnya barah payudara secara amnya adalah reseptor estrogen positif, sebahagian daripadanya akan menjadi reseptor estrogen negatif. Reseptor estrogen positif payudara adalah berkait dengan prognosis yang lebih baik berbanding dengan reseptor estrogen negatif kerana ianya lebih bergerakbalas terhadap terapi hormon.

Bendalir sista payudara (BCF) diketahui kaya dengan hormon-hormon steroid dan faktor-faktor pertumbuhan. Bahan-bahan ini mungkin mempunyai kesan yang bererti ke atas pertumbuhan sel-sel epitelium mammary dan metabolisme estrogen di dalam payudara dan mungkin, memainkan peranan penting dalam patofisiologi dan perkembangan barah payudara. Estron sulfatase bertindak sebagai reservoir yang besar bagi estrogen-estrogen yang aktif di dalam payudara dan ditukar kepada estron oleh enzim estrone sulfatase Tujuan kajian ini adalah untuk menilai kesan BCF ke atas

dan aktiviti estron sulfatase di dalam reseptor estrogen positif MCF-7 dan reseptor estrogen negatif MDA-MB-231 titisan sel barah payudara dan untuk menentukan berat molekul protin-protin/ peptida-peptida di dalam BCF yang bertanggungjawab terhadap perencatan aktiviti estron sulfatase.

Pertumbuhan sel-sel MCF-7 dan MDA-MB-231 adalah direncat secara bererti oleh BCF. Aktiviti estron sulfatase telah direncat secara bererti di dalam sel-sel MDA-MB-231 sementara aktiviti estron sulfatase dirangsang di dalam sel MCF-7 oleh BCF. Aktiviti estron sulfatase secara bererti telah direncat oleh kedua-dua BCF terdialisis dan dialisat.

Kehadiran perencat-perencat endogen estron sulfatase di dalam BCF adalah penting kerana perencat-perencat ini adalah agen-agen yang berpotensi dalam rawatan barah payudara bersandar hormon. Sebatian-sebatian yang hadir di dalam BCF yang mempunyai berat molekul lebih kurang 28 kDa mungkin bertanggungjawab dalam perencatan pertumbuhan dan perebakan kanser payudara.

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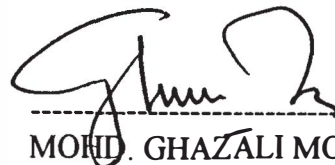
I certify that an Examination Committee met on 7th September 2000 to conduct the final examination of Rasha El-Hag El-Sadig on her Master thesis entitled “Effect of Breast Cyst Fluid on Oestrone Sulphatase Activity in Breast Cancer Cell Lines” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



RASHA EL-HAG EL-SADIG

Date: 11. 9. 2000

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LIST OF ABBREVIATIONS

ACS	American Cancer Society
APS	Ammonium persulphate solution
BCF	Breast cyst fluid
BCSC	Breast Cancer Society of Canada
cpm	Counts per minute
Da	Dalton
dpm	Disintegrations per minute
E ₂	Oestradiol
E ₂ DH	Oestradiol-17 β hydroxysteroid dehydrogenase
E ₁	Oestrone
ER ⁺	Oestrogen receptor-positive
ER ⁻	Oestrogen receptor-negative
E ₁ STS	Oestrone sulphatase
E ₁ S	Oestrone sulphate
EGF	Epidermal growth factor
FBS	Foetal bovine serum
GCDFP-70	Gross cystic disease fluid protein-70
GCDFP-44	Gross cystic disease fluid protein-44
GCDFP-24	Gross cystic disease fluid protein-24
GCDFP-15	Gross cystic disease fluid protein-15
HPLC	High performance liquid chromatography
IL-1	Interleukin-1
IL-6	Interleukin-6
SDS-PAGE	Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis
PBS	Phosphate buffered saline
PR ⁺	Progesterone receptor-positive
PR ⁻	Progesterone receptor-negative
PRE	progesterone response element
TGF- α	Transforming growth factor- α
TGF- β	Transforming growth factor- β



CHAPTER I

INTRODUCTION

Breast cancer represents 30% of all cancers in women and accounts for 20% of deaths due to cancer (Pasqualini *et al.*, 1995). Breast cancer is the second most important cancer killer in the world. It occurs most commonly in postmenopausal women when the ovaries have stopped producing oestrogens.

Breast cancer, like other cancers, is a disease of abnormal growth of cells. The epithelial cells, which line the milk producing ducts in the breast are the cells that typically become cancerous and form a solid mass of epithelial cells. These cells undergo a controlled cycle of growth and death during each menstrual cycle. The growth of these cells is stimulated by the action of steroid hormones such as oestrogen and progesterone. Breast cancer cells may metastasise to the lymph nodes, bones, liver, lungs and brain.

There are several risk factors that have been associated with increased incidence of breast cancer. Some, like a person's age or race, can not be changed.

Others are linked to cancer-causing factors in the environment. Still others are related to personal choices such as diet. Oestrogens have been shown to play an important role in promoting breast cancer (American Cancer Society, ACS).

Oestrogen is a stimulator of breast epithelial cell proliferation in normal breast. Most breast tumours are oestrogen dependent for growth in the early stages. A large proportion of breast cancer subsequently become oestrogen receptor-negative. The mechanism of this conversion is still not completely understood. In antioestrogen therapy the drug occupies the oestrogen receptor sites, which leads to artificially low oestrogen receptor values. In hormone dependent cells the association of the hormone with the receptor molecule is the basic step for eliciting a hormone response (Pasqualini *et al.*, 1995). In cancer cells the receptor gene can mutate leading to non-functioning oestrogen receptors which result in cells failing to respond to hormone treatment.

In the menopausal years the main source of oestrogen production is adipose tissue but normal and malignant breast tissues can also produce oestrogens (Reed, 1995). Three enzyme complexes are involved in oestrogen synthesis in peripheral tissues (Figure 1):

1. The aromatase enzyme complex, which converts androstenedione, secreted mainly by the adrenal cortex, to the weak oestrogen, oestrone (E_1).

2. Oestradiol-17 β hydroxysteroid dehydrogenase (E_2DH), which in breast cancer tissue acts preferentially to convert oestrone (E_1) to the more potent oestrogen, oestradiol (E_2).
3. Oestrone sulphatase (E_1STS), which hydrolyses the large amounts of oestrone sulphate (E_1S) formed from oestrone E_1 , back to oestrone E_1 .

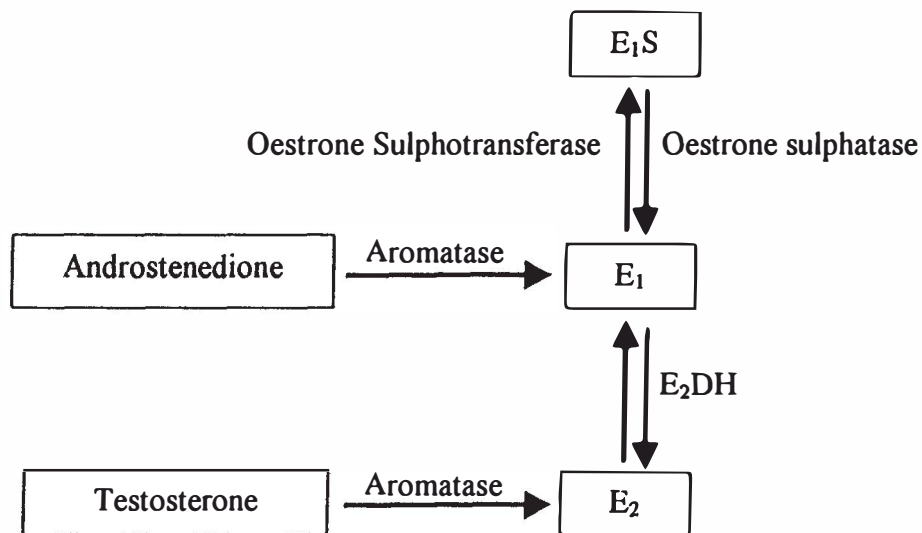


Figure 1: Enzymes involved in oestrone synthesis in normal and malignant breast tissues.

Adapted from Reed M.J, Molecular Medicine Today, 1: 98-103, 1995.

In human breast tumours, the sulphatase pathway is more important than the aromatase pathway, because the amount of oestrone produced from oestrone sulphate is 10 times higher than oestrone formed from androstenedione (Santner *et al.*, 1984). Thus, it has been suggested that E_1S acts as a huge reservoir for oestrogen formation (Purohit *et al.*, 1995).

Breast cysts are the most common benign lesions of the female breast. In the Western World 7% of women are affected. Breast cysts occur mainly between the ages of 35 and 50 (Dixon *et al.*, 1983). Breast cysts produce fluid which may vary in colour from yellow to green to brown. Occasionally the fluid may be bloody. Breast cyst fluid (BCF) is rich in sex steroids including oestrone sulphate, and mitogenic growth factors. BCF has been found to significantly inhibit the oestrone sulphotase pathway in the oestrone receptor positive human breast cancer cell line, MCF-7. The oestrone sulphotase pathway has been shown to be stimulated in the oestrone receptor negative human breast cancer cell line, MDA-MB-231 (Erbas *et al.*, 1996).

AIMS

The aims of the present study were:

1. To confirm that BCF inhibits oestrone sulphotase activity in the MCF-7 cell line and stimulates oestrone sulphotase activity in the MDA-MB-231 cell line.
2. To determine the molecular weights of the proteins/peptides in BCF responsible for the inhibition of oestrone sulphotase activity in the MCF-7 cell line.

CHAPTER II

LITERATURE REVIEW

2.1 Epidemiology of Breast Cancer

Breast cancer is a major public health problem worldwide. Breast cancer comprises almost one third of all new cancer cases and 20% of cancer deaths in women are due to breast cancer. In 1993 in the United States 46,300 cases of breast cancer (300 males and 46,000 females) were recorded. It was estimated in 1997 that 181,600 new cases of breast cancer would be diagnosed in the United States and 44,190 people would die from the same disease during the same year (Hortobagyi, 1998). In 1930 the annual death rate from breast cancer in the United States was low but this annual death rate has continued to increase significantly since 1970 amongst women (Spratt *et al.*, 1995).

The highest mortality rate in the world from breast cancer was found in the United States and England and the lowest mortality rate was found in Japan and Thailand (Spratt *et al.*, 1995). On the other hand, the Japanese living in the United States have a higher mortality rate from breast cancer than the Japanese living in Japan. In addition, Japanese born in the United States have a higher mortality rate from breast cancer than Japanese immigrants.

In African and Asian countries the number of breast cancer patients is slightly higher in premenopausal women than in postmenopausal women. This is in contrast to the Western countries where only a third of patients were premenopausal women (Yip and Ng, 1996; Natarajan *et al.*, 1988; Chiedozi, 1985).

2.1.1 Breast Cancer in Malaysia

Among Malaysian and Singaporean women breast cancer is the leading cause of cancer deaths (Yip and Ng, 1996). In Peninsular Malaysia, the death rate from breast cancer has continued to increase significantly since 1982. Figure 2.1 shows that the highest mortality was found in Chinese, followed by Malays and Indians (Yip and Ng, 1996).

Malay women in Malaysia and Singapore have a lower incidence of breast cancer compared to Chinese and Indian women. This may be related to different lifestyles and a host of other socio-cultural differences amongst the races (Yip and Ng, 1996).

A group of Malaysian scientists have obtained profiles of breast cancer patients seen at the Breast Clinic, Kuala Lumpur Hospital (Nair *et al.*, 1995). In 1994, a total of

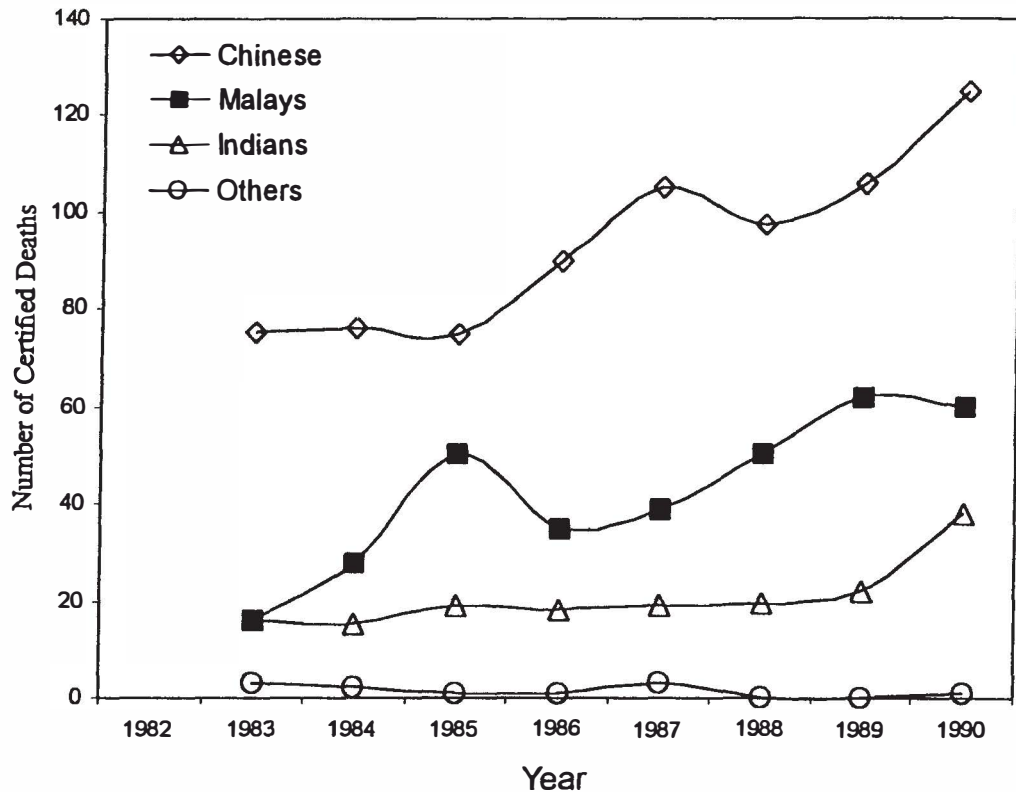


Figure 2.1: Certified deaths from breast cancer by race.
Adapted from Yip and Ng., Singapore Med J., 37: 264-267.

177 new breast cancer patients were seen; 40 patients (22.6%) were below the age of 40 years, 44% of patients were Malay, 32% were Chinese, 21% were Indians and 3% were of other ethnic groups. In 1995, a total of 169 new cases were seen at the same Hospital (K.L. Hospital); 21 patients (22.5%) were below the age of 40 years; 44% of the patients were Malays, 37% Chinese, 17% Indians and 2% belonging to other ethnic groups (Table 2.1)

A series of 125 cases of breast carcinoma at Kuantan General Hospital was studied. Overall the mean age of women was 48 years, being slightly younger (46 years) in Malays compared to Chinese (50 years). Only 42% of their patients presented